Obviousness-type double patenting.

Claims 17-19 were provisionally rejected under the judicially created doctrine of obviousness type double patenting as allegedly unpatentable over claims 1-62 of U.S. Patent No. 6,011,148. Applicants believe the Examiner meant to reject claims 18-20 because each of these dependent claims add the element "ultrafiltration" to claim 1. Upon notification of allowable subject matter, Applicants will file a terminal disclaimer, disclaiming any term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term defined in 35 U.S.C. §§ 154-156 and § 173 of prior Patent No. 6,011,148 issued January 4, 2000, thereby obviating the obviousness-type double patenting rejection.

35 U.S.C. § 103(a).

Claims 1-17 were rejected under 35 U.S.C. § 103(a) as allegedly obvious over WO 96/02658 ("Lee") in view of U.S. Patent No. 5,837,529 ("Wan") and Maniatis, T. *Molecular Cloning, Cold Spring Harbor Laboratory, pages 86-91, 1982* ("Maniatis"). In particular, the Examiner alleges that Lee teaches a method for purifying at least 100 mgs of plasmid DNA for pharmaceutical use by first heat lysing the cell mass, adding a precipitating agent, removing the precipitated component by centrifugation, ultrafiltering the solution and passing the clarified solution over an ion exchange column. The Examiner admits that Lee does not teach or suggest the use of an alkaline lysis agent, neutralizing agent nor static mixers.

The Examiner alleges that Wan teaches the use of an alkaline lysis agent, a neutralizing agent and static mixers for the preparation of plasmids. The Examiner further alleges that Maniatis teaches that heat lysis is equivalent to alkaline lysis of the cell mass in plasmid purification. The Examiner alleges that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the precipitation, centrifugation, ultrafiltration and ion exchange steps of Lee with the lysis, neutralization and static mixing steps of Wan to arrive at the claimed invention. Applicants respectfully traverse.

The Examiner is respectfully reminded that in order to find an invention *prima facie* obvious, the cited art must (1) teach or suggest each of the elements of the claimed invention, (2) provide suggestion or motivation to combine or modify the references, and (3) provide a reasonable expectation that one could successfully arrive at the claimed invention. See M.P.E.P. § 2143 *et seq.* Applicants submit that the Examiner has failed to make his *prima facie* case because the references in combination do not teach each and every element of the claimed invention, there is no suggestion or motivation to combine the references cited and there is no reasonable expectation of success.

The basic elements of the invention are defined in claim 1 as follows:

> A method for purifying plasmid DNA suitable for pharmaceutical use from bacterial cells on a large scale, the method comprising the following steps:
>
> a) contacting bacterial cells which together comprise at least about 100 milligrams of the plasmid DNA with a lysis solution, thereby forming a lysis mixture;
>
> b) flowing the lysis mixture through a first static mixer to obtain a lysed cell solution;
>
> c) contacting the lysed cell solution with a precipitation solution;
>
> d) flowing the lysed cell solution and the precipitation solution through a second static mixer, thereby forming a precipitation mixture;
>
> e) centrifuging the precipitation mixture, thereby forming a pellet and a clarified solution comprising the plasmid DNA; and
>
> f) neutralizing either the precipitation mixture prior to the centrifugation of step (e) or the clarified solution following the centrifugation of step (e);
>
> g) contacting the clarified solution with a positively charged ion exchange chromatography resin, wherein the plasmid DNA is eluted from the ion exchange chromatography resin with a saline step or continuous gradient; thereby producing a solution of plasmid DNA of sufficient purity and quantity for pharmaceutical use, wherein the solution comprises at least about 100 mg of the plasmid DNA.

The prior art cited by the Examiner neither teaches or suggests the neutralization step required by claim 1, step (f). Applicants teach adding a neutralizing agent to either the precipitation solution prior to centrifugation or to the resulting clarified solution following centrifugation (see step (f) of claim 1) to help preserve the integrity of the plasmid DNA during the purification process.

The Examiner relies on Wan for teaching the neutralization step, which is an element of the claimed invention. As the Examiner admits on page 4, second paragraph of the Office Action, "WO 96/02656 [Lee] did not teach the use of an alkaline lysis agent, neutralization agent, nor static mixers." The Examiner alleges that Wan teaches these three elements of the claimed invention. Applicants respectfully submit that the Examiner has mischaracterized Wan. The Examiner has pointed to nothing in the Wan patent that refers to either an implicit or explicit neutralization step.

The deficiency in the teachings of Wan is not found in the other references cited by the Examiner. As stated *supra*, the Examiner admits that Lee does not teach the neutralization step. This step is also not found in the portions of Maniatis of record. The Examiner cited Maniatis for teaching that alkaline and heat lysis are equivalent. The Examiner was silent about any other teachings in the reference. The Examiner has identified nothing in this reference that discusses the relative merits of neutralization and alkali lysis.

Applicants submit that the Examiner has not established a *prima facie* case of obviousness because the Examiner has not cited references which teach all of the elements of the claimed invention. Applicants respectfully request that the 35 U.S.C. § 103(a) rejection of claims 1-17 be withdrawn.

Assuming *arguendo* that the cited art did teach or suggest all of the elements of the claimed invention, the Examiner has failed to make his *prima facie* case of obviousness because there is no suggestion or motivation to combine or modify the references cited in a manner indicated by the Examiner. As stated by the Court of Appeals for the Federal Circuit:

> Our case law makes it clear that the best defense against hindsight-based obviousness analysis is the rigorous application of the requirement for a showing of a teaching or motivation to combine the prior art references. See *Dembiczak,* 175 F.3d at 999, 50 USPQ2d at 1617. "**Combining prior art references without evidence of such suggestion, teaching, or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability--the essence of hindsight." Id** [emphasis added] *Ecolochem, Inc. v Southern-California Edison Co.,* 227 F.3d 1361, 1371 (Fed. Cir. 2000)
>
> * * *
>
> The mere fact that the prior art may be modified in the manner suggested by the Examiner **does not** make the modification obvious unless the prior art suggested the desirability of the modification. [emphasis added] *In re Fritch*, 23 USPQ2d 1780, 1783-1784 (Fed. Cir. 1992)

As stated *supra* the presently pending claims consist of a unique combination of steps for the purification of pharmaceutical grade plasmid DNA that is not found in the cited references. The Examiner states that one of skill in the art would have been motivated to combine the precipitation, centrifugation, ultrafiltration and ion exchange methods of Lee with the lysis, neutralization and static mixing steps of Wan because Maniatis teaches that heat and alkali lysis are equivalent. Applicants respectfully disagree.

The Examiner made Maniatis of record in Paper No. 8, mailed January 4, 2000. In that Office Action, the Examiner specifically cited the teachings found on pages 86, 87, 90, and 91 of Maniatis. Pages 86 and 87 deal generally with procedures for isolating plasmid DNA and purifying the plasmid DNA away from the chromosomal DNA. Maniatis states that plasmid purification protocols exploit one of two important differences between chromosomal DNA and plasmid DNA, size (chromosomal DNA is large, plasmid DNA is small) and shape (chromosomal DNA is extracted as broken linear molecules, plasmid DNA is extracted as

covalent closed circular molecules) (page 86, paragraph 2). Maniatis teaches that both alkaline lysis and heat lysis purification protocols take advantage of these differences to separate plasmid DNA from chromosomal DNA.

In the instant Office Action, the Examiner has not provided any additional guidance for where Maniatis teaches that heat and alkali lysis protocols are equivalent. Treating bacterial cells with either heat or alkali may achieve the same goal of lysing cells and denaturing DNA, however, each method may have different consequences.

In fact, Lee actually teaches away from the equivalency of heat and alkaline lysis by teaching away from using alkaline lysis for large scale plasmid purification. Lee states, "[t]he classical techniques for isolating plasmid DNA from microbial fermentations are suitable for small or laboratory scale plasmid preparations. One such procedure involves the alkaline lysis of microbial host cells containing the plasmid, followed by acetate neutralization causing the precipitation of host cell genomic DNA which are then removed by, for example, centrifugation." See Lee page 1, lines 5-10. Lee further states:

> There are numerous drawbacks and limitations to this process including:
> a) the process involves the use of expensive and hazardous chemicals . . .;
> b) the density centrifugation step is not easily scaleable;...
> f) **scalability of the chemical lysis step is an obstacle *i.e.*, lysosyme, alkaline/KOAc treatment step is efficient in lysing cells on a small scale, however, the increase in viscosity makes large scale processing very difficult**; . . . [emphasis added] See Lee page 1, line 24-page 2, line 14.

In addition to teaching that the alkaline lysis procedure discussed *supra*, is not suitable for pharmaceutical/industrial scale plasmid purification, Lee suggests that other small or laboratory scale plasmid purification schemes, *i.e.,* those taught by Maniatis, are not suitable for high volume plasmid purification. For example, Lee states that the Holmes and Quigley method for purifying plasmid is only suitable for very small scale preparations (see Lee, page 2, line 28 - page 3, line 9.

In summary, Lee does not teach or suggest that alkaline lysis and potassium acetate precipitation are suitable techniques for pharmaceutical/industrial scale plasmid purification and actually teaches away from using these techniques. Based on the teachings of Lee, one of skill in the art would not be motivated to combine the ultrafiltration and anion exchange techniques of Lee with the lysis, precipitation and static mixer steps taught by Wan to arrive at the claimed invention.

In view of the foregoing remarks, Applicants submit that the Examiner has not established a *prima facie* case of obviousness because the cited references do not teach all of the

elements of the claimed invention, there is no suggestion or motivation to combine the cited references and there is no reasonable expectation that one of skill in the art would arrive at the claimed invention. Applicants respectfully request that the 35 U.S.C. § 103(a) rejection of claims 1-17 be withdrawn.

Claims 1-20 were rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over WO 96/02658 (Lee) in view of U.S. Patent No. 5,837,529 (Wan) and Maniatis and further in view of U.S. Patent No. 5,256,294 ("van Reis") and U.S. Patent No. 5,034,314 ("Geiger"). In particular, the Examiner alleges that Lee, Wan and Maniatis teach a method of purifying plasmid DNA as discussed above. The Examiner admits that Lee, Wan and Maniatis do not teach purifying a nucleic acid from a solution by ultrafiltering the solution through an ultrafilter comprising a gel layer which retains the nucleic acid, thereby purifying the nucleic acid. The ultrafilter may be in an open-channel, flat plate or hollow fiber device.

The Examiner alleges that van Reis teaches that gel layers are inherent in all ultrafiltration processes and that the process can be performed in dead ended, tangential flow (with or without screens) and hollow fiber devices. The Examiner further alleges that Geiger teaches practical ranges of operation of a tangential flow ultrafiltration device for purification of DNA. The Examiner alleges that it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the method of purifying a nucleic acid from a solution using an ultrafiltration system which retains the nucleic acid creating a gel layer with the practical knowledge of the parameters which can be used to separate nucleic acid species by ultrafiltration. Applicants respectfully traverse.

As discussed above, in order to find an invention *prima facie* obvious, the cited art must (1) teach or suggest each of the elements of the claimed invention, (2) provide suggestion or motivation to combine or modify the references, and (3) provide a reasonable expectation that one could successfully arrive at the claimed invention. Applicants submit that the Examiner has failed to make his *prima facie* case because, as discussed above, Lee, Wan and Maniatis in combination do not teach or suggest all of the elements of the claimed invention, fail to provide any suggestion or motivation to combine the steps which are disclosed in those references and fail to provide any reasonable expectation for arriving at the invention claimed. In addition, there is no suggestion or motivation to combine the teachings of van Reis and Geiger with those of Lee, Wan and Maniatis and one of skill in the art would not have a reasonable expectation of success at arriving at the claimed invention based on these additional references.

The instant application discloses the desirability of purifying plasmid DNA by inclusion of a filtration step involving passing solutions containing plasmid DNA through an ultrafilter on which a gel layer composed of plasmid DNA has formed. See *e.g.* specification page 13, lines 7-11 and page 13, line 22 - page 14, line 20. The presence of the gel layer during the ultrafiltration process results in higher yields and greater purity of the plasmid being isolated. The Examiner relies on van Reis for the teaching that gel layers are inherent in the process of ultrafiltration and alleges that this makes the instant invention obvious. The Examiner is reminded, "[a] retrospective view of inherency is no substitute for some teaching or suggestion which supports the selection and use of the various elements in the particular claimed combination." *In re Newell* 891 F.2d 899 (Fed. Cir. 1989). Applicants state that van Reis does not provide any teaching or suggestion supporting the benefits of a gel layer in the ultrafiltration process for plasmid DNA preparations. van Reis does not deal with nucleic acid preparations at all and explicitly teaches away from these benefits indicating that gel layers or their synonyms, concentration polarization or fouling, limit the utility of ultrafiltration for purifying macromolecules.

van Reis states "[h]owever, limitations exist on the degree of protein purification achievable in ultrafiltration. These limits are due mainly to the phenomena of concentration polarization, fouling and wide membrane pore size distribution." See van Reis, column 1, lines 61-65. van Reis states further:

> **A result of concentration polarization and fouling processes is the inability to make effective use of the macromolecular fractionation capabilities of ultrafiltration membranes for the large-scale resolution of macromolecular mixtures** such as blood plasma proteins. . . . Consequently, the potentially exciting utilization of membrane ultrafiltration for large-scale complex macromolecular mixture-separations . . . is considered elusive. [emphasis added] See van Reis, column 2, lines 16-31.

Based on these teachings of van Reis, one of skill in the art would not be motivated to develop a gel layer during an ultrafiltration step to aid in plasmid DNA purification.

As stated above, the Examiner relies on the teaching of van Reis "the polarized layer can never be completely eliminated" (see van Reis, column 4, lines 36-37) to state that the instant invention is obvious because gel layers are inherent to the ultrafiltration process. However, the full sentence containing the phrase on which the Examiner relies teaches away from the desirability of having the gel layer "[d]espite all of these attempts at improvement, it is still the case that although concentration polarization can be modified, and quite high filtration rates can be achieved even from very concentrated solutions if appropriate flow conditions are supplied, the polarization layer can never be completely eliminated." See van Reis, column 4,

lines 31-37. As indicated by the Court of Appeals for the Federal Circuit, even if an element is inherent in a process, there must be a teaching or suggestion which supports the use of the element in the particular claimed combination. Nothing in van Reis teaches or suggests that gel layers are beneficial in an ultrafiltration step to aid in plasmid DNA purification.

Furthermore, as previously mentioned, van Reis is directed toward protein purification, not DNA purification. Although van Reis does suggest that ultrafiltration can be used for DNA purification, the reference does not teach any conditions which would be suitable for plasmid DNA purification. More importantly, van Reis teaches that the object of the instant application, forming gel layers to aid purification of biological macromolecules should be minimized or eliminated. As the Examiner acknowledges on page 7 last line of the office action, van Reis states, "[i]t is another object to provide improved filtration processes, including ultrafiltration processes, for separating biological macromolecules such as proteins which processes **minimize concentration polarization** and do not increase flux." [emphasis added] See van Reis, column 4, lines 60-64. van Reis cites attempts in the art "[t]o circumvent the effects of concentration polarization several processes have been developed that modify the feed plasma source to improve selectivity and flux." See van Reis, column 2, lines 43-45. Examples of such processes that minimize concentration polarization cited by van Reis include: sample dilution (column 2, lines 48-53); changing fluid flow to move retained solute away from the membrane surface (column 2, lines 60-68); and creating pressure differentials to increase solute flow through the filter (column 3, lines 9-12 and lines 35-48). Again, one of skill in the art, faced with the teachings of van Reis, would conclude that developing a gel layer during the ultrafiltration step in a plasmid DNA purification protocol should be avoided.

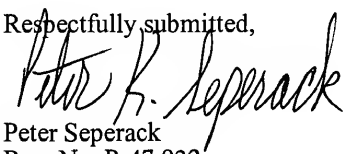
The Examiner relies on Geiger for teaching "practical knowledge as to the various parameters which can be used to effect separation of nucleic acid species by ultrafiltration" (office action, page 8). Geiger is directed toward separating double stranded nucleic acid molecules from a mixture of double and single stranded nucleic acids including single stranded nucleic acid probes. Geiger teaches separating μgm amounts of nucleic acid in μl volumes of solution by ultrafiltration in the laboratory setting. One of skill in the art would appreciate that the amount of nucleic acid present in the solution taught by Geiger would be insufficient to form a gel layer during the filtration process. Geiger does not teach or suggest that a gel layer forms during the ultrafiltration step. Geiger does not teach or suggest that it would be advantageous to have a gel layer form to aid in the separation of the single and double stranded nucleic acid species. Geiger does not teach or suggest that his methods would be applicable to the purification of bulk quantities of plasmid DNA.

In summary, the Examiner has combined references which do not teach or suggest each of the elements of the claimed invention, which teach away from using alkali lysis for pharmaceutical/industrial plasmid DNA purification, and which teach away from purifying plasmid DNA by ultrafiltration through a gel layer. None of the references cited by the Examiner teach or suggest the combination of steps disclosed and claimed by the Applicants in the instant application. As discussed above, stating that one of skill in the art would arrive at the claimed invention by combining the cited references in the manner taught by the Examiner is at best taking the Applicants' disclosure "as a blueprint for piecing together the prior art to defeat patentability -- the essence of hindsight". Applicants submit that the Examiner has not established a *prima facie* case of obviousness because the cited references do not teach or suggest all of the elements of the claimed invention, there is no suggestion or motivation to combine the cited references and there is no reasonable expectation that one of skill in the art would arrive at the claimed invention using the teachings of the cited references. Applicants respectfully request that the 35 U.S.C. § 103(a) rejection of claims 1-20 be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is urged. If the Examiner believes a telephone conference would aid in the prosecution of this case in any way, please call the undersigned at 415-576-0200.

Respectfully submitted,



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MARKED UP VERSION OF THE CHANGES TO THE CLAIMS

IN THE CLAIMS:

21. (New) A method for purifying plasmid DNA suitable for pharmaceutical use from bacterial cells on a large scale, the method comprising the following steps:

a) contacting bacterial cells which together comprise at least about 100 milligrams of the plasmid DNA with a lysis solution, thereby forming a lysis mixture;

b) flowing the lysis mixture through a first static mixer to obtain a lysed cell solution;

c) contacting the lysed cell solution with a precipitation solution;

d) flowing the lysed cell solution and the precipitation solution through a second static mixer, thereby forming a precipitation mixture;

e) centrifuging the precipitation mixture, thereby forming a pellet and a clarified solution comprising the plasmid DNA; and

f) neutralizing either the precipitation mixture prior to the centrifugation of step (e) or the clarified solution following the centrifugation of step (e);

g) contacting the clarified solution with a positively charged ion exchange chromatography resin, wherein the plasmid DNA is eluted from the ion exchange chromatography resin with a saline step or continuous gradient; and

h) filtering the clarified solution of step (f) through an ultrafiltration unit comprising a gel layer either before or after contacting the clarified solution with the positively charged ion exchange resin of step (g), thereby producing a solution of plasmid DNA of sufficient purity and quantity for pharmaceutical use, wherein the solution comprises at least about 100 mg of the plasmid DNA.

APPENDIX A – PENDING CLAIMS

1. A method for purifying plasmid DNA suitable for pharmaceutical use from bacterial cells on a large scale, the method comprising the following steps:

a) contacting bacterial cells which together comprise at least about 100 milligrams of the plasmid DNA with a lysis solution, thereby forming a lysis mixture;

b) flowing the lysis mixture through a first static mixer to obtain a lysed cell solution;

c) contacting the lysed cell solution with a precipitation solution;

d) flowing the lysed cell solution and the precipitation solution through a second static mixer, thereby forming a precipitation mixture;

e) centrifuging the precipitation mixture, thereby forming a pellet and a clarified solution comprising the plasmid DNA; and

f) neutralizing either the precipitation mixture prior to the centrifugation of step (e) or the clarified solution following the centrifugation of step (e);

g) contacting the clarified solution with a positively charged ion exchange chromatography resin, wherein the plasmid DNA is eluted from the ion exchange chromatography resin with a saline step or continuous gradient; thereby producing a solution of plasmid DNA of sufficient purity and quantity for pharmaceutical use, wherein the solution comprises at least about 100 mg of the plasmid DNA.

2. The method of claim 1, further comprising the step of RNase digestion.

3. The method of claim 1, wherein the lysis solution contains alkali.

4. The method of claim 1, wherein the precipitation solution contains potassium acetate.

5. The method of claim 1, wherein the neutralizing step precedes the step of centrifuging the precipitation mixture.

6. The method of claim 1, wherein the linear velocity of the lysis mixture through the first static mixer is between about 0.38 to 2.3 feet per second and the first static mixer has an outer diameter in the range of from about 3/16" inch to about 2 inches.

7. The method of claim 6, wherein the first static mixer has 24 elements.

8. The method of claim 6, wherein the first static mixer is a laminar flow static mixer.

9. The method of claim 1, wherein the linear velocity of the precipitation mixture through the second static mixer is between 0.38 to 2.3 feet per second and the second static mixer has an outer diameter in the range of from about 3/16 inch to about 2 inches.

10. The method of claim 9, wherein the second static mixer is a laminar flow static mixer.

11. The method of claim 9, wherein the second static mixer has 24 elements.

12. The method of claim 1, wherein steps (a) and (b) are carried out simultaneously.

13. The method of claim 1, wherein steps (c) and (d) are carried out simultaneously.

14. The method of claim 1, wherein steps (a), (b), (c), and (d) are carried out simultaneously.

15. The method of claim 1, wherein steps (a), (b), (c), (d) and (e) are carried out simultaneously.

16. The method of claim 1, wherein steps (a), (b), (c), (d) (e) and (f) are carried out simultaneously.

17. The method of claim 16, wherein the method is automated.

18. The method of claim 1, further comprising filtering the clarified solution through an ultrafiltration unit comprising a gel layer before contacting the clarified solution with the positively charged ion exchange resin.

19. The method of claim 18, wherein the ultrafiltration unit comprises a membrane having a molecular weight cutoff of from about 50K to about 500K daltons. 20.

20. The method of claim 1, further comprising ultrafiltration of the plasmid DNA using tangential flow ultrafiltration with an open channel device, in the presence of a gel layer.

21. (New) A method for purifying plasmid DNA suitable for pharmaceutical use from bacterial cells on a large scale, the method comprising the following steps:

a) contacting bacterial cells which together comprise at least about 100 milligrams of the plasmid DNA with a lysis solution, thereby forming a lysis mixture;

b) flowing the lysis mixture through a first static mixer to obtain a lysed cell solution;

c) contacting the lysed cell solution with a precipitation solution;

d) flowing the lysed cell solution and the precipitation solution through a second static mixer, thereby forming a precipitation mixture;

e) centrifuging the precipitation mixture, thereby forming a pellet and a clarified solution comprising the plasmid DNA; and

f) neutralizing either the precipitation mixture prior to the centrifugation of step (e) or the clarified solution following the centrifugation of step (e);

g) contacting the clarified solution with a positively charged ion exchange chromatography resin, wherein the plasmid DNA is eluted from the ion exchange chromatography resin with a saline step or continuous gradient; and

h) filtering the clarified solution of step (f) through an ultrafiltration unit comprising a gel layer either before or after contacting the clarified solution with the positively charged ion exchange resin of step (g), thereby producing a solution of plasmid DNA of sufficient purity and quantity for pharmaceutical use, wherein the solution comprises at least about 100 mg of the plasmid DNA.